Establishment of dexamethasone model as a model for metabolic-associated hepatic injury in male Wistar rats

Ahmed E. Amer[1,2,*], Hamdy A. Ghoneim[2], Rania R. Abdelaziz[2], George S. G. Shehatou[1,2], Ghada M. Suddek[2]

[1] Department of Pharmacology and Biochemistry, Faculty of Pharmacy, Delta University for Science and Technology, International Coastal Road, Gamasa City, Dakahliya, 11152, Egypt

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

*Correspondence: Ahmed E. Amer; Delta University for Science and Technology, International Coastal Road, Gamasa City, Dakahliya, 11152, Egypt; Email, Ph_ahmedamer@yahoo.com; Phone number, +201007844698

ABSTRACT

This study aimed to establish a dexamethasone model as a representative model for metabolic-associated hepatic injury in male Wistar rats. dexamethasone is a synthetic glucocorticoid with potent anti-inflammatory effects and high glucocorticoid activity. The model was developed to induce insulin resistance and nonalcoholic fatty liver disease in a relatively short period.

Male Wistar rats were allotted into three groups: control, DEX8, and DEX16. The rats of DEX8 and DEX16 groups were injected intraperitoneally with dexamethasone for 6 days at doses of 8 mg/kg/day and 16 mg/kg/day, respectively. Fasting blood glucose levels were measured, and various biochemical parameters were analyzed. Liver sections were collected and examined for histopathological changes.

The results showed that dexamethasone administration significantly increased fasting blood glucose levels compared to the control group. Liver indices were also significantly elevated in the dexamethasone-administered groups. Moreover, serum alanine aminotransferase and aspartate aminotransferase activities were significantly increased in the dexamethasone groups, indicating liver damage. Histopathological examination revealed hydropic degeneration, portal edema, leukocyte infiltration, and fibrosis in the liver sections of dexamethasone-treated rats. These findings demonstrate that the dexamethasone model successfully induced metabolic-associated hepatic injury in male Wistar rats, as evidenced by hyperglycemia, altered liver indices, increased liver enzymes, and histopathological changes resembling NASH. The model provides a valuable tool for studying the pathophysiology of metabolic liver diseases and evaluating potential therapeutic interventions. It offers a convenient and time-efficient approach to investigate the effects of dexamethasone and develop strategies to mitigate its adverse effects on liver function.

Keywords:

Dexamethasone ; NASH ; Insulin resistance

1. Introduction

Glucocorticoids (GC) are a type of steroid commonly employed for their anti-inflammatory effects in various clinical conditions, including asthma and rheumatoid arthritis. Additionally, GC serve as immunosuppressor in organ transplantation patients. The broad range of clinical applications for GC highlights their significance in managing inflammatory and immune-related disorders (Kumar *et al.*, 2015).

When the body experiences acute stress or a decrease in glucose intake, the endogenous glucocorticoid hormone cortisol is released into the bloodstream. This physiological response is aimed at meeting the body's demand for glucose. Cortisol acts by promoting gluconeogenesis, the process of creating new glucose molecules from non-carbohydrate sources such as amino acids and fatty acids. By increasing blood glucose levels, cortisol ensures an adequate energy supply to meet the body's requirements during times of stress or limited glucose availability (Patel *et al.*, 2011).

Dexamethasone is a frequently prescribed GC that exhibits significantly higher potency in its glucocorticoid activity compared to the naturally occurring cortisol hormone (Du *et al.*, 2015).

Several studies used dexamethasone to induce insulin resistance in whole tissues of rodents (Severino *et al.*, 2002; Patel *et al.*, 2011). Moreover, it was shown that dexamethasone administration to male Wistar rats resulted in the development of nonalcoholic fatty liver disease (NAFLD) (Vinodraj *et al.*, 2015). Furthermore, increased triglycerides (TG) in the liver of dexamethasone-administered rats was reported (Patel *et al.*, 2011; Barbosa *et al.*, 2016). Additionally, it was shown that treating rats with dexamethasone resulted in marked increases in the levels of TG and cholesterol (Mahendran and Devi, 2001). It has been shown that dexamethasone administration resulted in glucose intolerance, hyperglycemia, impaired insulin sensitivity, dyslipidemia, and hepatic steatosis with associated increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (El-Sonbaty *et al.*, 2019).

Furthermore, it has been recently shown that severe damage and fatty degeneration in both hepatic and renal tissues was developed by dexamethasone administration, which is combined with significant elevations in serum ALT, AST, alkaline phosphatase (ALP), urea, blood glucose, creatine kinase (CK)-MB, lactate dehydrogenase (LDH), TG, total cholesterol, and oxidative stress (Ahmed *et al.*, 2020). Moreover, Intraperitoneal administration of dexamethasone (4,8 and 16mg/kg) for 6 days developed fatty changes in the livers of experimental rats (Kumar *et al.*, 2015).

In line with this, it was reported that dexamethasone administration resulted in marked lipid accumulation in the liver with elevation of glucose levels, insulin, lipid, liver volume, and liver weight (Vinodraj *et al.*, 2015; Nayak *et al.*, 2017). The main benefit of using dexamethasone models is that they can develop insulin resistance in rodents in a relatively short period (Okumura *et al.*, 1998; Korach-Andre *et al.*, 2005; Kumar *et al.*, 2015).

Taken together, this study was undertaken to establish the model of dexamethasone as described earlier by Kumar *et al.* (2015) to induce metabolic-associated hepatic injury in Wistar rats.

2. Material and methods

2.1. Drugs

Dexamethasone was utilized as dexamethasone sodium phosphate ampules (Amirya, Egypt).

2.2. Experimental animals

Nine Wistar rats (Males, 250-270 g) were supplied from VACSERA research center (Helwan, Egypt). They were allowed to be accommodated in the Animal House of the Faculty of Pharmacy, Delta University for 10 days before induction of the experiment. They were kept under standard conditions and handled according to the guidelines of the research ethics committee of the Faculty of Pharmacy, Delta University for Science and Technology.

2.3. Experimental design

Following the protocol that has been described early by Kumar *et al.* (2015), nine male Wistar rats (250-270 g) were allotted into three groups (each of 3 rats), as follows: control group, rats were subjected to injection of saline (16 mg/kg/day, i.p.); DEX8 group, rats were injected with dexamethasone (8 mg/kg/day, i.p.) for 6 days; DEX16 group, rats were injected with dexamethasone (16 mg/kg/day, i.p.). After 6 days of dexamethasone administration, fasting blood glucose was measured, rats were anesthetized by intraperitoneal thiopental (50 mg/kg) (Amer *et al.*, 2021; Amer *et al.*, 2022), blood was collected from the orbital sinus for later biochemical analysis, and rats were sacrificed by decapitation. Later, liver sections were collected, dried, and weighed for determination of liver indices.

2.4. Fasting blood glucose measurement

Rats were subjected to overnight fasting on the 5th day of dexamethasone administration. On the morning of the 6th day of dexamethasone administration, each rat's fasting blood glucose was measured from a blood sample taken by cutting the tail using CareSens® electronic glucometer.

2.5. Assay of serum ALT and AST activities

Serum ALT (Cat.no. 12212, Human diag.) respectively and AST (Cat. No. 12211, Human diag.) activities were determined using commercially available kits according to the supplier's instructions.

2.6. Histopathology

After dewaxing and hydration, 5 μm slices of left lobes of hepatic tissue were stained with H&E and evaluated by a professional pathologist under a light microscope to detect any pathological changes.

2.7. Statistical analysis

Mean ± standard deviation was used to demonstrate the data. Data was analyzed occupying one-way analysis of variance (ANOVA) and Tukey post hoc test. Statistical significance is typically determined by the probability value (*P* value) of less than 0.05.

3. Results

3.1. Fasting blood glucose levels

Compared to the control group, DEX8 and DEX16 groups showed significantly higher fasting blood glucose levels by 2.62 and 2.74 folds, respectively (*P*<0.05, **Figure 1**).
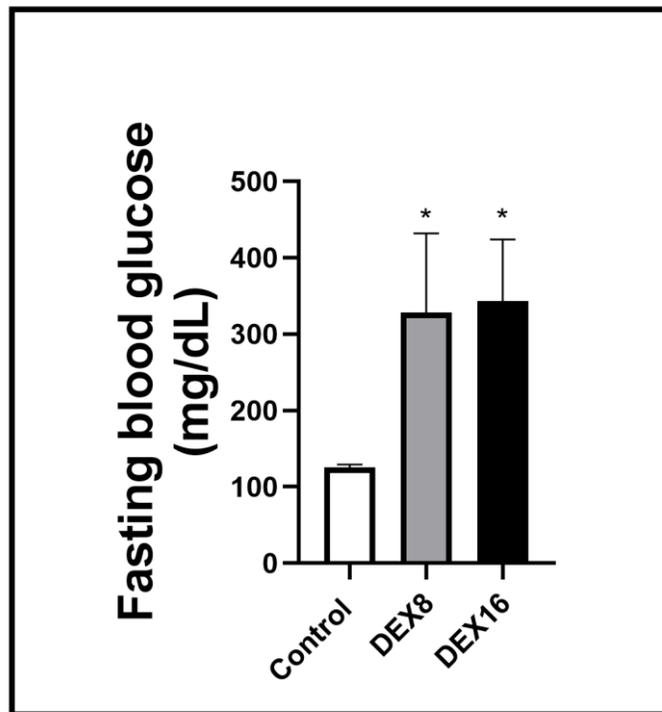
Figure 1. Impact of dexamethasone (8 and 16 mg/kg/day) on fasting blood glucose

Data are displayed as Mean ± SD. *significant from the control group at *P* < 0.05, using one way ANOVA.

3.2. Liver indices

Relative to the control group, the administration of dexamethasone (8 and 16 mg/kg/day) resulted in a marked increase in liver indices by 2.10 and 2.13 folds ($P < 0.01$), respectively (**Figure 2**).
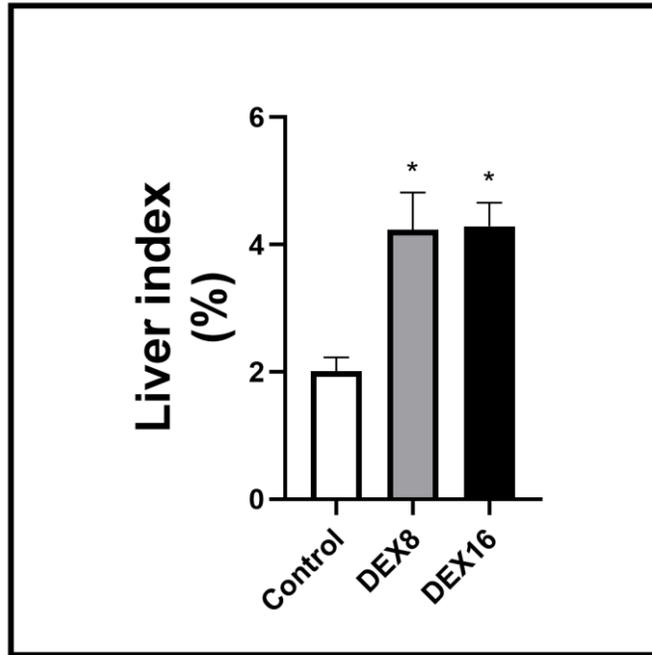
Figure 2. Impact of dexamethasone (8 and 16 mg/kg/day) on liver indices

Data are displayed as Mean ± SD. *significant from the control group at $P < 0.05$, using way ANOVA.

3.3. Serum ALT and AST

DEX8 group showed considerable raises in serum ALT and AST activities by 3.80 ($P < 0.05$) and 3.30 ($P < 0.01$), respectively, relative to the control group (Figure 3A and B). Moreover, injection of dexamethasone 16 mg/kg/day resulted in marked elevations of serum ALT and AST activities by 4.58 and 3.44 folds, respectively, relative to the control rats ($P < 0.01$**, Figure 3A and B**).
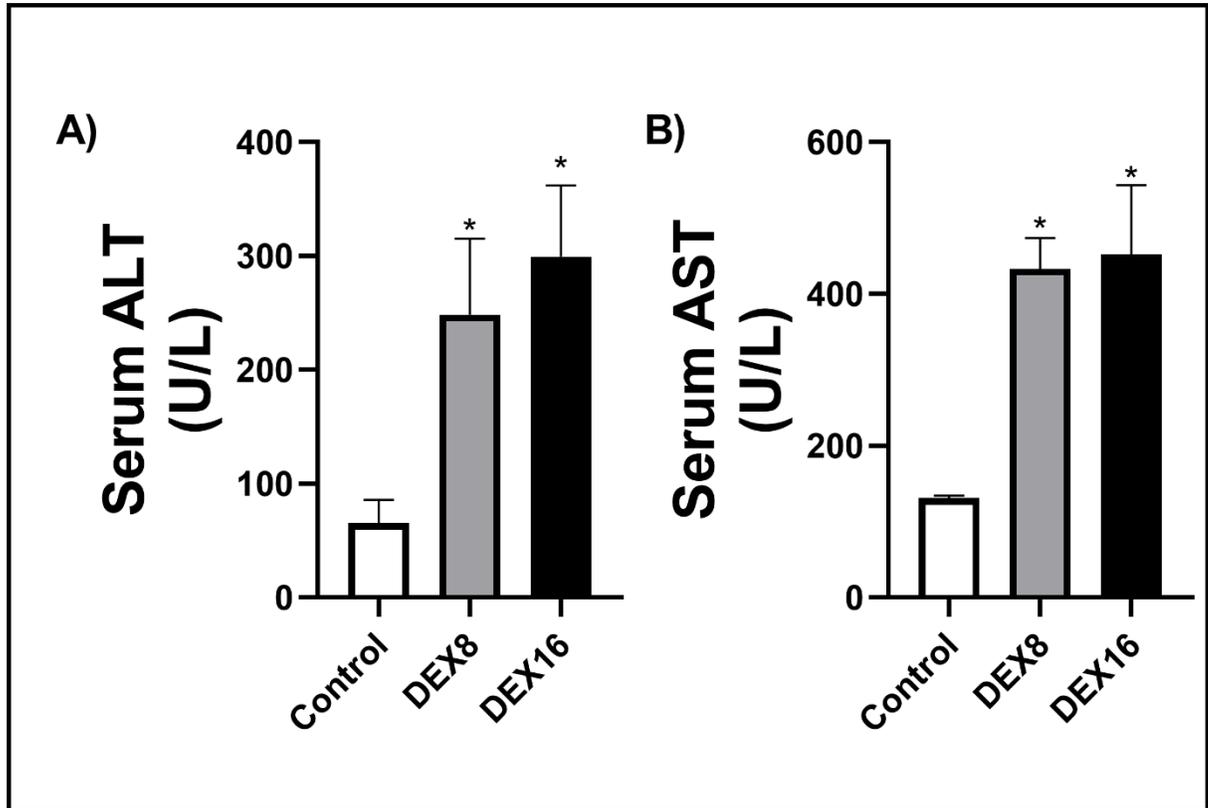
Figure 3. Impact of dexamethasone (8 and 16 mg/kg/day) on serum ALT and AST activities

A. Serum ALT activity, B. Serum AST activity. Data are displayed as Mean ± SD. *significant from the control group at $P < 0.05$, using way ANOVA. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.

3.4. Morphological and histopathological changes

Figure 4 demonstrates representative H&E-stained sections from the different groups. Sections from the control group showed normally organized hepatic cords, normal portal area (PA), normal central veins (CV), and normal sinusoids (S). Liver sections from DEX8 group exhibited multifocal areas of hydropic degeneration (black arrowheads) in hepatocytes, congested central veins (red arrows), portal edema with few leukocytes cells infiltration (black arrows), portal fibrosis (yellow arrows) with occluded sinusoids. Liver sections from DEX16 group showed increased hydropic degeneration (black arrowheads) to be diffuse in hepatocytes, congested portal veins (red arrows), and portal fibrosis (yellow arrows) with occluded sinusoids.
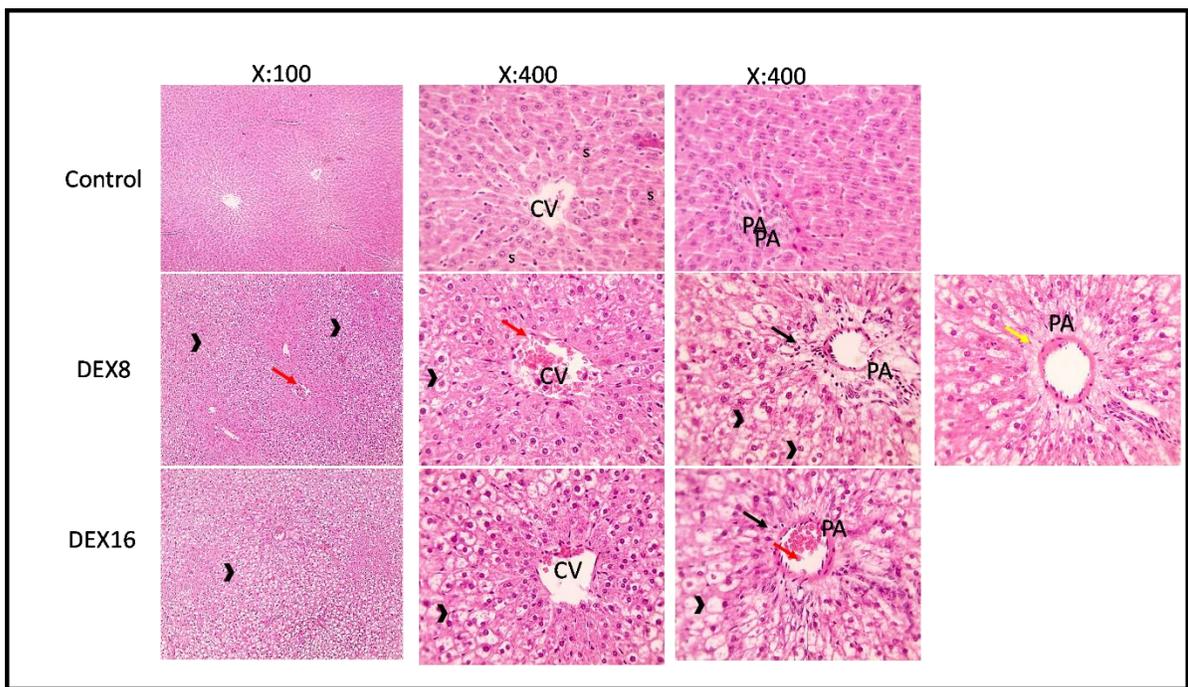
Figure 4. Impact of dexamethasone (8 and 16 mg/kg/day) on histopathological features

The section of the control group shows normally organized hepatic cords and normal portal area (PA), central veins (CV), and sinusoids (S) while the section from DEX8 group shows multifocal areas of hydropic degeneration (black arrowheads) in hepatocytes, congested central veins (red arrows), portal edema with few leukocytes cells infiltration (black arrows), portal fibrosis (yellow arrows) with occluded sinusoids. DEX16 group's section shows increased hydropic degeneration (black arrowheads) to be diffuse in hepatocytes, congested portal veins (red arrows), and portal fibrosis (yellow arrows) with occluded sinusoids. PA, Portal area; CV, central veins; S, Sinusoids

Discussion

Dexamethasone is a potent synthetic GC with a high affinity for GC receptors **(El-Sonbaty *et al.*, 2019)**. The mechanism underlines this cortisol action depends on increasing hepatic glucose synthesis (gluconeogenesis) and decreasing peripheral glucose uptake into muscle and adipose tissue **(Patel *et al.*, 2011; Kumar *et al.*, 2015)**. The main drawbacks of using therapeutic GC for a prolonged time are hyperglycemia and insulin resistance, which may finally govern the progression of NAFLD **(Du *et al.*, 2015)**.

In the current study, dexamethasone administration (8 and 16 mg/kg/day) for 6 days resulted in considerable metabolic and hepatic consequences including hyperglycemia, increased liver indexes, and alteration of serum liver function markers. These consequences are in line with the previous findings of **Kumar *et al.* (2015).** Furthermore, dexamethasone administration showed significant pathological alterations in the histological features of the liver. These alterations include ballooning degenerations, inflammation, and fibrosis, mimicking the nature of nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis is the advanced stage of nonalcoholic fatty liver disease which is strongly correlated to metabolic abnormalities including diabetes mellitus.

Tissue insulin sensitivity may be decreased in several ways in response to GC exposure. Insulin signaling pathways are downregulated by GC. GC inhibit the transcription of IRS-1 in mouse skeletal muscle. Simultaneously, they increase the transcription of two proteins that counter insulin action, protein tyrosine phosphatase 1B (PTP1B) and p38 mitogen-activated protein kinase **(Almon *et al.*, 2005)**. Moreover, GC inhibit IRS-1 phosphorylation in the liver and reduce IRS-1 and IRS-2 levels in adipose tissue **(Bazuine *et al.*, 2004; Rahimi *et al.*, 2020)**. Also, GC stimulate the transcription of genes encoding gluconeogenic enzymes such as G6Pase and phosphoenolpyruvate carboxy kinase (PEPCK) to enhance gluconeogenesis in the liver and kidney **(Lochhead *et al.*, 2000; Zhang *et al.*, 2019)**. Moreover, both hyperglycemia and hyperinsulinemia, which are induced by GC, stimulate the activation of sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP). SREBP-1c and ChREBP are responsible for *de novo* fatty acids synthesis **(Ferre and Foufelle, 2010; Filhoulaud *et al.*, 2013; Rahimi *et al.*, 2020)**.

Furthermore, lipolysis in subcutaneous adipose tissue is stimulated by GC while they induce hypertrophy and differentiation of adipocytes in visceral adipose tissue, in line with the phenotype of Cushing's syndrome patients **(Shibli-Rahhal *et al.*, 2006; Vegiopoulos and Herzig, 2007; Chanson and Salenave, 2010)**. GC' actions on lipolysis lead to increased levels of released free fatty acids (FFAs) into circulation **(Rahimi *et al.*, 2020)**.

FFAs are taken by tissues such as the heart, skeletal muscle, and liver by fatty acid transporters **(Alves-Bezerra and Cohen, 2017)**. CD36, a well-studied fatty acid transporter, is expressed at a higher level in the liver of GC-treated rats **(D'souza *et al.*, 2012)**. FFAs are then converted in the liver to TG and stored as lipid droplets **(Dolinsky *et al.*, 2004; Rahimi *et al.*, 2020)**. GC Stimulate VLDL secretion while they inhibit plasma lipoprotein activity **(Rahimi *et al.*, 2020)**.

Taken together, Plasma TG levels are strongly increased by GC as reported previously (**Dolinsky *et al.*, 2004; Choi and Ginsberg, 2011**). Moreover, GC suppress beta-oxidation of fatty acids as a result of their inhibition of the transcriptional activity of peroxisome proliferator-activated receptor (PPAR) -α (**Marino *et al.*, 2016**). PPARα is a key mediator of the expression of mitochondrial acyl-CoA dehydrogenases, which are responsible for the beta-oxidation of FFAs (**Vega *et al.*, 2000; Leone *et al.*, 2005**). Collectively, GCs-stimulation of lipolysis in the presence of elevated plasma levels of FFAs and stimulation of FFAs uptake by the liver is the predominant mechanism of hepatic lipid accumulation (**Rahimi *et al.*, 2020**).

Conclusion

In conclusion, the current study observed significant metabolic and hepatic consequences following the administration of dexamethasone in rats. These consequences included hyperglycemia, increased liver indices, and alterations in serum liver function parameters, consistent with previous findings. Furthermore, the histological analysis revealed pathological changes in the liver, resembling features of nonalcoholic steatohepatitis. However, no appreciable changes were observed between the dose of 8 mg/kg/day and 16 mg/kg/day of dexamethasone. Therefore, the dose of 8 mg/kg/day will be appropriate for the induction of these consequences to study the potential therapeutic options in future studies. Moreover, we reviewed some of the possible potential mechanisms that may underlie the hepatic consequences of dexamethasone which may guide possible mechanistic targets of future studies.

Disclosure
The authors declare no conflict of interest.

References

Ahmed, E.I., Shaaban, A.M., Abdel Latif, A.K.M., 2020. Effect of Canagliflozin, an SGLT2 Inhibitor, in Comparison with Atorvastatin on Dexamethasone-Induced Hepatic Steatosis in Albino Rats. Current Drug Therapy **15**, 274-282.

Almon, R.R., DuBois, D.C., Jin, J.Y., Jusko, W.J., 2005. Temporal profiling of the transcriptional basis for the development of corticosteroid-induced insulin resistance in rat muscle. Journal of endocrinology **184**, 219-232.

Alves-Bezerra, M., Cohen, D.E., 2017. Triglyceride metabolism in the liver. Comprehensive Physiology **8**, 1.

Amer, A.E., El-Sheakh, A.R., Hamed, M.F., El-Kashef, H.A., Nader, M.A., Shehatou, G.S.G., 2021. Febuxostat attenuates vascular calcification induced by vitamin D3 plus nicotine in rats. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences **156**, 105580.

Amer, A.E., Shehatou, G.S.G., El-Kashef, H.A., Nader, M.A., El-Sheakh, A.R., 2022. Flavocoxid Ameliorates Aortic Calcification Induced by Hypervitaminosis D(3) and Nicotine in Rats Via Targeting TNF-α, IL-1β, iNOS, and Osteogenic Runx2. Cardiovascular drugs and therapy **36**, 1047-1059.

Barbosa, A.M., Francisco, P.d.C., Motta, K., Chagas, T.R., Dos Santos, C., Rafacho, A., Nunes, E.A., 2016. Fish oil supplementation attenuates changes in plasma lipids caused by dexamethasone treatment in rats. Applied Physiology, Nutrition, and Metabolism **41**, 382-390.

Bazuine, M., Carlotti, F.o., Tafrechi, R.S.J., Hoeben, R.C., Maassen, J.A., 2004. Mitogen-activated protein kinase (MAPK) phosphatase-1 and-4 attenuate p38 MAPK during dexamethasone-induced insulin resistance in 3T3-L1 adipocytes. Molecular endocrinology **18**, 1697-1707.

Chanson, P., Salenave, S., 2010. Metabolic syndrome in Cushing's syndrome. Neuroendocrinology **92**, 96-101.

Choi, S.H., Ginsberg, H.N., 2011. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. Trends in Endocrinology & Metabolism **22**, 353-363.

D'souza, A.M., Beaudry, J.L., Szigiato, A.A., Trumble, S.J., Snook, L.A., Bonen, A., Giacca, A., Riddell, M.C., 2012. Consumption of a high-fat diet rapidly exacerbates the development of fatty liver disease that occurs with chronically elevated glucocorticoids. American Journal of Physiology-Gastrointestinal and Liver Physiology **302**, G850-G863.

Dolinsky, V.W., Douglas, D.N., Lehner, R., Vance, D.E., 2004. Regulation of the enzymes of hepatic microsomal triacylglycerol lipolysis and re-esterification by the glucocorticoid dexamethasone. Biochemical Journal **378**, 967-974.

Du, W.W., Liu, F., Shan, S.W., Ma, X.C., Gupta, S., Jin, T., Spaner, D., Krylov, S.N., Zhang, Y., Ling, W., 2015. Inhibition of dexamethasone-induced fatty liver development by reducing miR-17-5p levels. Molecular Therapy **23**, 1222-1233.

El-Sonbaty, Y.A., Suddek, G.M., Megahed, N., Gameil, N.M., 2019. Protocatechuic acid exhibits hepatoprotective, vasculoprotective, antioxidant and insulin-like effects in dexamethasone-induced insulin-resistant rats. Biochimie **167**, 119-134.

Ferre, P., Foufelle, F., 2010. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. Diabetes, obesity and metabolism **12**, 83-92.

Filhoulaud, G., Guilmeau, S., Dentin, R., Girard, J., Postic, C., 2013. Novel insights into ChREBP regulation and function. Trends in Endocrinology & Metabolism **24**, 257-268.

Korach-Andre, M., Gao, J., Gounarides, J., Deacon, R., Islam, A., Laurent, D., 2005. Relationship between visceral adiposity and intramyocellular lipid content in two rat models of insulin resistance. American Journal of Physiology-Endocrinology and Metabolism **288**, E106-E116.

Kumar, V.H., Im, N.N., Huilgol, S.V., Yendigeri, S.M., K, N., Ch, R., 2015. Dose Dependent Hepatic and Endothelial Changes in Rats Treated with Dexamethasone. Journal of clinical and diagnostic research : JCDR **9**, Ff08-10.

Leone, T.C., Lehman, J.J., Finck, B.N., Schaeffer, P.J., Wende, A.R., Boudina, S., Courtois, M., Wozniak, D.F., Sambandam, N., Bernal-Mizrachi, C., Chen, Z., Holloszy, J.O., Medeiros, D.M., Schmidt, R.E., Saffitz, J.E., Abel, E.D., Semenkovich, C.F., Kelly, D.P., 2005. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS biology **3**, e101.

Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G., Sutherland, C., 2000. 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes **49**, 896-903.

Mahendran, P., Devi, C.S., 2001. Effect of Garcinia cambogia extract on lipids and lipoprotein composition in dexamethasone administered rats. Indian journal of physiology and pharmacology **45**, 345-350.

Marino, J.S., Stechschulte, L.A., Stec, D.E., Nestor-Kalinoski, A., Coleman, S., Hinds, T.D., 2016. Glucocorticoid Receptor β Induces Hepatic Steatosis by Augmenting Inflammation and Inhibition of the Peroxisome Proliferator-activated Receptor (PPAR) α*♦. Journal of Biological Chemistry **291**, 25776-25788.

Nayak, I.M.N., Narendar, K., M, P.A., Jamadar, M.G., Kumar, V.H., 2017. Comparison of Pioglitazone and Metformin Efficacy against Glucocorticoid Induced Atherosclerosis and Hepatic Steatosis in Insulin Resistant Rats. Journal of clinical and diagnostic research : JCDR **11**, Fc06-fc10.

Okumura, S., Takeda, N., Takami, K., Yoshino, K., Hattori, J., Nakashima, K., Sugimoto, M., Ishimori, M., Takami, R., Yasuda, K., 1998. Effects of troglitazone on dexamethasone-induced insulin resistance in rats. Metabolism **47**, 351-354.

Patel, R., Patel, M., Tsai, R., Lin, V., Bookout, A.L., Zhang, Y., Magomedova, L., Li, T., Chan, J.F., Budd, C., Mangelsdorf, D.J., Cummins, C.L., 2011. LXRβ is required for glucocorticoid-induced hyperglycemia and hepatosteatosis in mice. J Clin Invest **121**, 431-441.

Rahimi, L., Rajpal, A., Ismail-Beigi, F., 2020. Glucocorticoid-induced fatty liver disease. Diabetes, metabolic syndrome and obesity: targets and therapy **13**, 1133.

Severino, C., Brizzi, P., Solinas, A., Secchi, G., Maioli, M., Tonolo, G., 2002. Low-dose dexamethasone in the rat: a model to study insulin resistance. American journal of physiology. Endocrinology and metabolism **283**, E367-373.

Shibli-Rahhal, A., Van Beek, M., Schlechte, J.A., 2006. Cushing's syndrome. Clinics in dermatology **24**, 260-265.

Vega, R.B., Huss, J.M., Kelly, D.P., 2000. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Molecular and cellular biology **20**, 1868-1876.

Vegiopoulos, A., Herzig, S., 2007. Glucocorticoids, metabolism and metabolic diseases. Molecular and cellular endocrinology **275**, 43-61.

Vinodraj, K., Nagendra Nayak, I.M., Rao, J.V., Mathai, P., Chandralekha, N., Nitasha, B., Rajesh, D., Chethan, T.K., 2015. Comparison of the efficacy of liraglutide with pioglitazone on dexamethasone induced hepatic steatosis, dyslipidemia and hyperglycaemia in albino rats. Indian journal of pharmacology **47**, 181-184.

Zhang, X., Yang, S., Chen, J., Su, Z., 2019. Unraveling the regulation of hepatic gluconeogenesis. Frontiers in endocrinology **9**, 802.